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Genomics and array technology.

DeRisi JL, Iyer VR

Brown Lab, Department of Biochemistry, Stanford University Medical Center, Palo Alto, CA 94305, USA.

Genome projects are providing vast amounts of sequence data. This raw material makes possible a completely new era of experimental approaches. Among these, DNA array technology, which allows one to assay thousands of unique nucleic acid samples simultaneously, will be important in genomic research, and the results of this research are likely to affect virtually every field of biology. DNA array technology is still in its infancy, but many have demonstrated its power by using it for such diverse applications as global monitoring of gene expression, mutation detection, and genetic mapping.

Publication Types:
- Review
- Review, tutorial

PMID: 9914882, UI: 99113311

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Revised: April 3, 2000.

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Sensitivity issues in DNA array-based expression measurements and performance of nylon microarrays for small samples.

Bertucci F, Bernard K, Loriod B, Chang YC, Granjeaud S, Birnbaum D, Nguyen C, Peck K, Jordan BR

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DNA or oligonucleotide arrays are widely used for large-scale expression measurements, using various implementations: macroarrays in which DNA is spotted onto nylon membranes of relatively large dimensions (with radioactive detection) on the one hand; microarrays on glass slides and oligonucleotide chips, both used with fluorescent probes, on the other hand. Nylon micro-arrays with colourimetric detection have also been described recently. The small physical dimensions of miniaturized systems allow small hybridization volumes (2-100 microl) and provide high probe concentrations, in contrast to macroarrays. We show, however, that actual sensitivity (defined as the amount of sample necessary for detection of a given mRNA species) is in fact similar for all these systems and that this is mostly due to the very different amounts of target material present on the respective arrays. We then demonstrate that the combination of nylon microarrays with(33)P-labelled radioactive probes provides 100-fold better sensitivity, making it possible to perform expression profiling experiments using submicrogram amounts of unamplified total RNA from small biological samples. This has important implications in basic and clinical research and makes this alternative approach particularly suitable for groups operating in an academic context.

PMID: 10441335, UI: 99371779

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1 : *Gene* 1995 Apr 24;156(2):207-13

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High-density cDNA filter analysis: a novel approach for large-scale, quantitative analysis of gene expression.

Zhao N, Hashida H, Takahashi N, Misumi Y, Sakaki Y

Human Genome Center, Institute of Medical Science, University of Tokyo, Japan.

In order to analyze the expression profiles of a large number of genes in the tissues (or cells) of interest, and to identify the genes preferentially expressed in the tissues, we have developed a large-scale gene expression analysis system. It is based on the hybridization of the mRNAs from the tissues with a high-density cDNA filter followed by the quantitative measurement of the amount of the hybridized mRNA on each cDNA spot. By employing a high-performance bioimaging analyzer, the system allowed us to compare the expression profiles of thousands of genes (cDNAs) simultaneously with a sensitivity comparable to conventional Northern blotting analysis. By this system (called high-density cDNA filter analysis or HDCFA), the expression profiles of 2505 cloned human brain cDNAs (genes) were monitored. Through the comparison of the expression profiles of these cDNAs in the adult brain, fetal brain and adult liver, about one half of these brain cDNAs (1239 clones) were identified as the candidates which were expressed preferentially in the brain. Among these, 408 and 288 clones were found to be preferentially expressed in the adult and fetal brain, respectively. The results have shown that the system may be widely applicable for analysis of the gene expression profiles of various tissues on a large scale.

PMID: 7758958, UI: 95278746

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Direct hybridization of large-insert genomic clones on high-density gridded cDNA filter arrays.

Kern S, Hampton GM

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A major challenge to positional cloning approaches is the identification of coding sequences within a region of interest. Hybridization of genomic fragments that represent a cloned contig of a defined genomic region in appropriate cDNA libraries theoretically represents a direct solution to this problem. However, this is technically difficult and in general, success with this approach has been limited to the use of small fragments, such as those cloned in cosmids and phages. Since most physical maps are composed of genomic DNA cloned in vectors with significantly greater insert size capacity, there is a need to develop efficient methods to use these clones directly as hybridization probes. Here we describe a highly sensitive protocol for hybridization of P1-derived artificial chromosomes (PACs; average insert size, 120 kb) on a composite, normalized cDNA library comprised of 200000 clones spotted at high density on nylon filters. Because limited sequence information on more than 150000 of these clones is now available in the public domain, positive hybridization results can be rapidly converted to cDNA sequence information without recourse to any clone manipulation in the initial phases of a project. Using these protocols, we have been able to reproducibly detect coding exons that constitute as little as 0.2% of the total PAC insert.

PMID: 9232242, UI: 97376070

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